The Relationship between Dynamic Compression and Gene Expression of Single Chondrocytes and Chondrons

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INTRODUCTION

Chondrocytes produce a hydrated pericellular matrix (PCM) which is rich in glycosaminoglycans, proteoglycan and distinct collagens; together they form a 'chondron' [1,2]. The precise function of the PCM is not known but it clearly has a major impact in regulating the biomechanical environment of the chondrocyte and influencing its activity [3]. The retention of the *in vivo* PCM has been reported to positively influence chondrocyte gene expression resulting in improved matrix production [4]. In this work, the effect of dynamic compression on gene expression of single chondrocytes and chondrons was investigated. The results may improve our understanding of how the PCM regulates its biomechanical environment and help improve current cartilage tissue engineering strategies.

Single chondrocytes and chondrons were compressed at two levels of deformation (20% & 40%) using micromanipulation [5]. The gene expression of several matrix components and transcription factors was quantified using a novel single cell real-time RT-PCR assay.

METHODS

Cell preparation: Full depth articular cartilage was dissected from the trochleal humerus of four 18-month-old cows. Both chondrocytes and chondrons were enzymatically isolated and fully characterised as previously described [2].

Single cell compression: Single cells (n=12) were selected and compressed between the flat end of a force probe made of glass fibre and the flat bottom surface of the chamber. Dynamic compression of either 20% or 40% cell deformation was applied at a frequency of 0.3Hz for 10min. After compression, the cell was collected with a microaspirator (30µm inner diameter; CellTram) and transferred to a tube. Single cells were either lysed immediately or incubated at 37°C for 18h and then lysed.

Single cell PCR: Single cells were lysed (Cells-to-cDNA™ lysis buffer) at 75°C in a thermal cycler for 10 min. Samples were treated with Dnase I to eliminate genomic DNA and then reverse transcribed (High Capacity cDNA RT Kits). cDNA were preamplified (TaqMan® PreAmp Master Mix Kit), diluted before performance of real-time PCR (ABS 7300 Real-Time PCR system). All bovine primers/probes used were designed by File Builder 3.1 software and manufactured by Applied Biosystems. The gene expression of matrix components (collagen type II & X, aggrecan, hyaluronan, lubricin, osteopontin) and transcription factors (Sox9, CFBA1 and NF KappaB) was quantitated.

Data analysis: The threshold cycle (C_T) for each sample was calculated based on the cycle number at which the peak of the second derivative of the fluorescence vs. cycle number (7300 System SDS Software 1.3.1). Efficiency of the PCR was calculated by running a standard curve for serially diluted cDNA (0.003µg/µl to 3µg/µl) isolated from bovine chondrocytes.

The relative abundances of all genes were calculated using a method adapted from Pfaffl [6]. In brief, the abundance, A, was calculated using the equation: $A = (1 + E)^{-CT}$, where E is reaction efficiency, C_T is the threshold cycle. To normalise the data, the abundance of all target genes were divided by the abundance of 18s gene in that cell.

A two-way ANOVA was used to determine the difference between chondrocytes and chondrons subjected to the same deformation. Results were considered statistically significant for $p<0.05$. Results are presented as means ± standard errors (n=12).

RESULTS

Measurable levels of at least one gene were detected in 96/96 cells tested (100%). Of these, 100% expressed 18s, 100% expressed COL2, 93.8% expressed AGG, 91.7% expressed lubricin, 88.5% expressed osteopontin, 77.1% expressed SOX9, 33.3% expressed CFBA1, 32.3% expressed NF KappaB, 20.1% expressed Has2, and 10.0% expressed COL10.

The control data for cells lysed immediately and cells lysed after 18h incubation were comparable therefore we have reported the mean o f

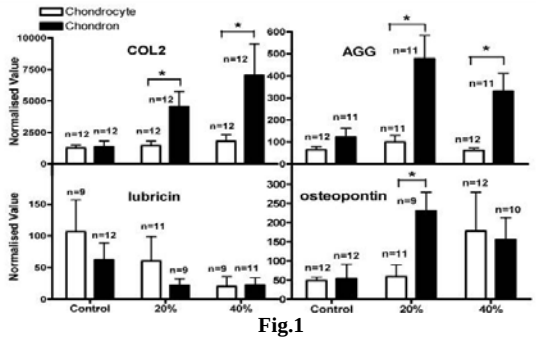

Fig.1

these control data. Fig.1. shows matrix gene expression for chondrons and chondrocytes after 20% and 40% deformation. Following their deformation, the chondrons had significantly higher levels of COL2 ($p=0.0154$, $p=0.00418$), AGG ($p=0.0028$, $p=0.0023$) and osteopontin ($p=0.0049$) gene expression than the chondrocytes

Fig. 2 shows transcription factor gene expression for chondrocytes and chondrons following 20% and 40% deformation. Chondrocytes expressed significantly higher levels of CBFA1 ($p=0.04$) than chondrons at 20% compression. There was no significant difference at 40% deformation.

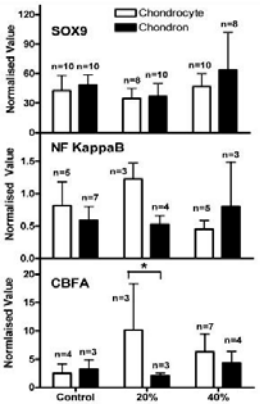

Fig.2

DISCUSSION

The components of the PCM are an important consideration for engineering articular cartilage [7]. We have demonstrated that the presence of the PCM influences the biological responses of the chondrocyte. The ratio of COL2 to AGG gene expression is similar to that found throughout all zones of articular cartilage. By comparison, lubricin and osteopontin gene expression were low. These results wereto be expected since they are both expressed in the superficial and deep zone, respectively. It is important to note that we used tissue from skeletally immature cows. SOX9 gene expression remained constant in both chondrocytes and chondrons during the entire experiment suggesting that there was no change in phenotype. CBFA1 gene expression is elevated in chondrocytes. NF KappaB was slightly elevated but the sample number was low so this result is inconclusive.

In conclusion, we have demonstrated that we can isolate, compress and assess gene expression of single cells. It will be interesting to study single chondrocytes and chondrons from different zones within articular cartilage.

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